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Induction of *In vitro* Flowering in *Arnebia hispidissima* (Lehm). DC.

M.S. Shekhawat

Department of Plant Biology and Biotechnology, K.M. Centre for P.G. Studies, Lawspet, Pondicherry, India
smahipal3@gmail.com

ABSTRACT:
In vitro flowering was induced in plantlets regenerated in cultures of *Arnebia hispidissima*. The nodal shoot segments were used as explants. These were surface sterilized and inoculated on Murashige and Skoog's (MS) agar-gelled medium + 2.0 mgl^{-1} 6-Benzylaminopurine (BAP) for bud breaking. The cultures were multiplied by passages on MS medium supplemented with 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} Indole-3 acetic acid (IAA). The elongated shoots produced on the same medium were excised and transferred to half strength MS medium supplemented with 1.0 mgl^{-1} indole-3 butyric acid (IBA). Rooting and flowering were observed on the 10th and 17th day after their transfer to rooting medium. Flower buds were developed from the plantlets under 12 h photoperiod and 65 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Shoots cultured under various media and photoperiods did not flower.

Key words: *In vitro*, flowering, *Arnebia hispidissima*, photosynthetic photon flux density.

INTRODUCTION

The switch from vegetative stage to reproductive stage is one of the most critical events in the life cycle of a plant. Studies on *in vitro* flowering using different light qualities, photoperiods and plant growth regulators have been reported for several plant species e.g. *Ocimum basilicum, Panax ginseng, Withania somnifera, Rauvolfia tetraphylla, Anethum graveolens, Solanum nigrum* etc. [1, 2, 3, 4, 5, 6]. Flowering obtained with *in vitro* cultures is a pre-requisite for many genetic manipulations and can also improve knowledge about physiology and molecular biology of flowering. The main advantages of *in vitro* flowering techniques are isolation of external signals from environment, acquisition of aseptic cultures and standardized conditions that permit repeating several cycles in a short period of time [7]. Additionally, *in vitro* cultures turn fast the studies about flowering.

The initiation of reproductive stage generally requires that plant perceives and responds to the appropriate environmental conditions [8]. Light is the most important environmental factor that induces changes in plant physiology and morphology, and regulating flowering cycles [9, 10, 11]. The application of cytokinins (especially BAP and Kinetin), sucrose concentrations, photoperiod, and subculture time to promote flowering *in vitro* is well documented in many plant species including roses [12,13].

Arnebia hispidissima, belongs to the family Boraginaceae, is found in desert regions of Rajasthan [14]. This is an erect or decumbent herb, clothed with long, white, bulbous-based hairs. It has alternate leaves, which are linear and lanceolate. Flowers are yellow colored with throat corolla. This plant is annual or perennial and growing in sandy dry soils.

Plant has very high drought and frost tolerances. The roots of *A. hispidissima* contain a red purple dye (alkannin) [15] which is used by the tribals for coloring their clothes. In addition, it has wide applications as a colorant in food, cosmetic and textiles industries. The alkannin and its optical isomer shikonin are root-specific secondary metabolites of *A. hispidissima* [16]. The alkannin has recently attracted the interest of researchers due to its anti-inflammatory, antimicrobial, antitumor activities and wound-healing properties [17].

An *in vitro* flowering system is considered to be a convenient tool to study specific aspects of flowering and the mechanisms of the reproductive process such as floral initiation, floral organ development and floral senescence [18]. The aim of the present investigation was to verify the effects of different growth regulators and photoperiods on *in vitro* flowering of *A. hispidissima*.

MATERIALS AND METHODS

The plants of *A. hispidissima* were collected from the selected sites of the Thar desert (India) during the months of November to January. Various types of explants like shoot segments, roots, axillary and terminal shoot apices were used for initiation of cultures. The explanting materials were initially washed with water containing 0.1% Bavistin for 10 minutes. These were surface sterilized with 90% ethanol for 50 seconds and then with 0.1% $HgCl_2$ solution for 5-6 minutes. Explants were then washed extensively with autoclaved water for 8-10 times.

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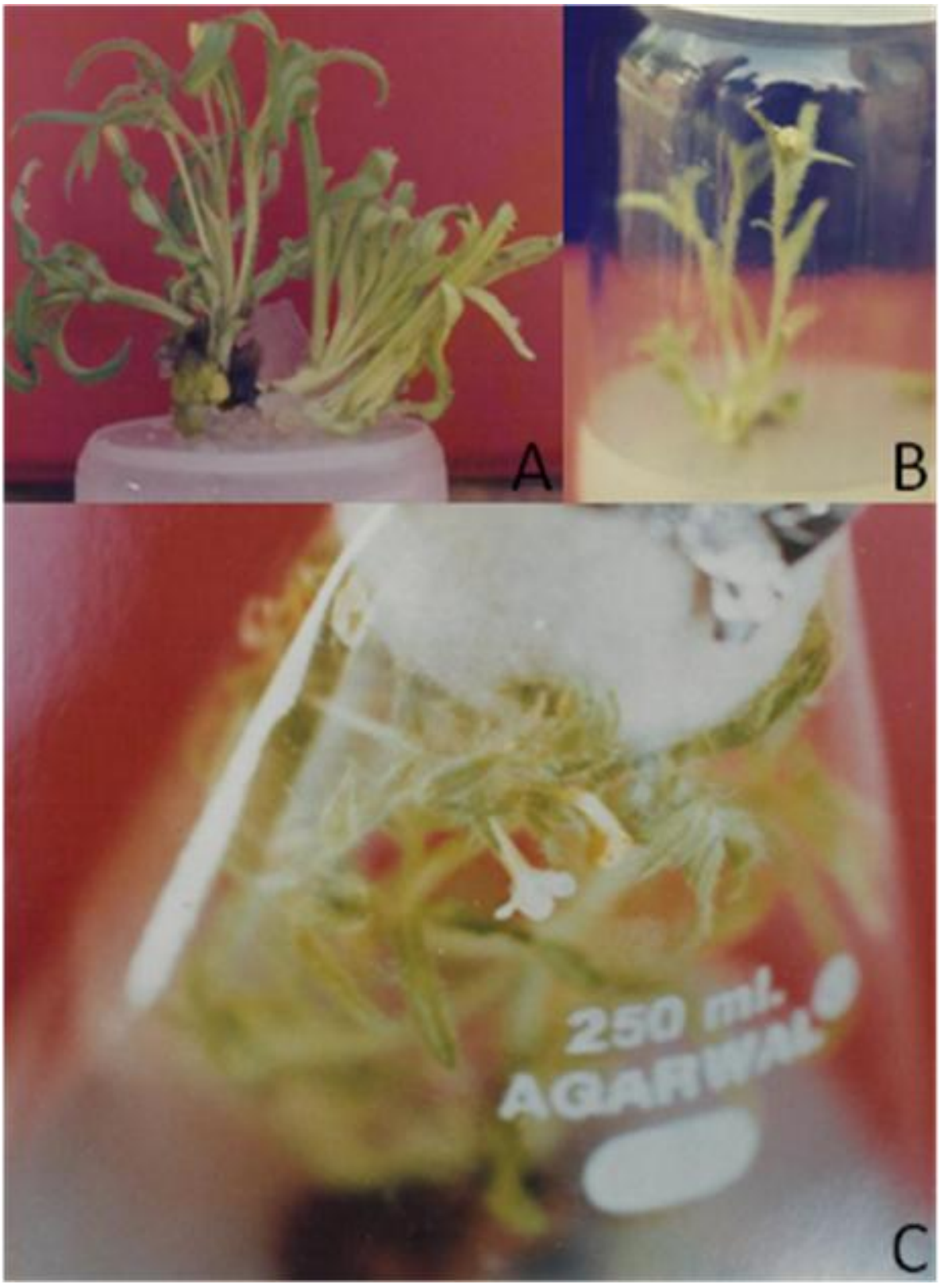

Figs. 1. A. Multiple shoots of *A. hispidissima* in cultures. 1. B. Initiation of flower. 1. C. *In vitro* regenerated plantlets with flowers.

FIGURE LEGENDS:

Figures: 1. A. Multiple shoots of *A. hispidissima* in cultures.
1. B. Initiation of flowering.
1. C. *In vitro* regenerated plantlets with flowers.

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Table 1: Effect of cytokinins on shoot multiplication and flower bud induction by shoot clump culture of *A. hispidissima* on MS medium containing 0.1 mgl^{-1} IAA.

Cytokinins (mgl^{-1})	Shoots number ± SD	Number of flower buds
BAP		
0.10	6.8±1.64	0
0.50	9.2±2.16	0
1.00	7.0±1.58	0
2.00	6.2±3.76	0
Kn		
0.10	3.0±1.58	0
0.50	4.4±1.14	0
1.00	4.0±1.58	0
2.00	3.2±0.83	0

Table 2: Effect of auxins and their concentrations on roots and flower buds induction from shoots of *A. hispidissima* on half-strength MS medium.

Auxin (mgl^{-1})	% of Response	Number of roots ± SD	Number of flower buds ± SD
IAA			
0.10	29	1.4±0.83	2.4±0.14
0.50	42	2.2±0.77	2.6±0.54
1.00	56	1.2±0.89	1.8±0.98
2.00	48	0.8±0.40	1.0±0.52
IBA			
0.10	31	2.4±0.44	2.0±0.46
0.50	63	2.6±0.73	2.6±0.31
1.00	85	3.2±0.54	3.6±0.63
2.00	76	1.8±0.33	2.4±0.10
NAA			
0.10	22	1.0±0.62	1.8±0.66
0.50	36	2.2±0.11	2.6±0.24
1.00	51	2.6±0.42	2.0±0.18
2.00	44	1.4±0.31	1.5±0.44

The surface sterilized explants were inoculated on MS medium [19]. Various concentrations of BAP, Kinetin (Kn) (1.0 to 5.0 mgl^{-1}) and IAA (0.1 to 1.0 mgl^{-1}) were incorporated in the culture media. To estimate the optimum conditions of temperature and light, these cultures were incubated at 25±2°C to 28±2°C temperature and 50-70 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) under 12-14 h photoperiod.

In vitro induced shoots were cut in 2-3 cm long segments, containing two to three nodes cultured on MS medium supplemented with various concentrations and combinations of auxins (IAA and NAA 0.1 to 1.0 mgl^{-1}) and cytokinins (BAP and Kn) ranging from 0.1 to 2.0 mgl^{-1}. Experiments were carried out to reduce the cost of production. Commercial sugar cubes and cheaper gelling agents were incorporated in the culture media.

In vitro regenerated shoots were harvested from the shoot clumps and were transferred to full strength and half strength MS medium. The medium was incorporated with various root inducing auxins (IAA, IBA and Naphthalene Acetic Acid) with variable concentrations (0.1 - 2.0 mgl^{-1}) along with 200 mgl^{-1} activated charcoal for root initiation and flower bud induction. Experiments were also conducted to induce flowering in *A. hispidissima* on various types of media. These were MS medium (full and half strength), Woody Plants medium [20], Whites medium [21] and B5 medium [22]. These media were incorporated with variable concentrations of BAP, Kn, IAA, IBA and NAA.

The observations were taken after every ten days of inoculation. The experiments were repeated thrice with ten replicates per treatment. The data were subjected to statistical analysis.

RESULTS AND DISCUSSION

Plant growth regulators represent important factors that regulate induction, evocation and development of flower buds. Cytokinins and auxins are associated with floral induction. Auxins, according to several studies, may either promote or inhibit flowering [23]. In many plants, *in vitro* flowering was normally achieved by the application of exogenous hormones to the culture medium [24].

Multiple shoots were induced when nodal segments were inoculated on MS media supplemented with different concentrations of BAP or Kn. The nodal segments cultured on media containing BAP (2.0 mgl^{-1}) alone had better potential for the multiple shoot induction than Kn alone. Superiority of BAP over other cytokinins for the shoot multiplication was reported in number of other medicinal plant species [25, 26, 27].

However, the number of shoots per explants increased significantly when IAA was added in BAP or Kn containing medium. MS medium containing 0.5 mgl^{-1} BAP and 0.1 mg l^{-1} IAA was most effective for shoot multiplication. It was observed that repeated or successive transfer of explants on this medium increased the number of shoots to 9.2 from 4.2 after 2-3 transfers (Table 1; Fig. 1A). The increase in shoot number may be due to suppression of apical dominance during subculture that induced basal dormant meristametic cells to form new shoots [29]. Flower bud formation was not observed at all at this stage of cultures in *A. hispidissima* at variable PPFD.

The role of various auxins (IAA, IBA and NAA) in the root induction and flower bud formation was also

tested in present investigated. Among different concentrations of three auxins tested, IBA (1.0 mgl^{-1}) was found to be better for the rooting as well as for flower bud initiation in shoots (Table 2). Auxins especially IBA is used widely to induce adventitious roots in many woody and herbaceous plant species [30, 31]. The present study clearly indicates that half-strength MS medium was adequate for the root induction and same time for flower bud formation. Relatively low salt concentration in the medium is known to enhance rooting of shoots [28]. Lower salt concentrations could also be helpful in induction of flower buds due to nutritional stress. Rooting and flowering were observed on the 10th and 17th day after their transfer to rooting medium on this media combination (Fig. 1B).

Out of various types of media tested for flower bud induction half strength MS medium was found most suitable. No flower bud formation was observed on other media and hormonal combinations even after 60 days of incubation on different PPFD. The maximum numbers of flower buds (3.6) formed at 65 µmol m^{-2} s^{-1} PPFD for12 h/d photoperiod (Fig. 1C). The results of the present investigation were contrary to the findings of Kanchanapoom et al. [31, 13]. They induced flowering in rose on MS medium supplemented with BA 2.0 mgl^{-1}.

In vitro flowering in *A. hispidissima* was demonstrated first time in present investigation. It may offer better understanding of nature of various factors which influences *in vitro* flowering in this plant.

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